Synaptojanin and Endophilin: Partners in Synaptic Transmission

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Endocytosis is a process by which cells uptake molecules by engulfing them with their membranes. It is a critical process for all eukaryotic cells, being involved in numerous processes including plasma membrane homeostasis, signal transduction, nutrient uptake, and pathogen entry. At neuronal synapses, endocytosis is specialized to sustain efficient neurotransmission by recycling neurotransmitter storage compartments called synaptic vesicles. Defects in synaptic endocytosis can lead to severe consequences, ranging from neuronal degeneration to lethality. While this indicates that vesicle recycling at synapses is critical for normal neural function, how it occurs is largely unknown. Two of the major players are the synaptojanin and endophilin proteins, and it is thought that the synaptojanin proline-rich domain (PRD) guides synaptojanin to endocytic sites through high-affinity interactions with the endocytic protein endophilin's src homology 3 (SH3) domain.

To investigate how synaptojanin and endophilin exert their positive effects on neurotransmission, postdoctoral fellow Dr. Yongming Dong and colleagues in the lab of Dr. Jihong Bai (Basic Sciences Division) studied mutant forms of synaptojanin in the nematode *Caenorhabditis elegans*. "The PRD-recruitment model has received a significant amount of attention over the years. However, we find that synaptojanin PRD is not required for synaptic activity *in vivo*. In fact, both the PRD and its binding partner (the endophilin SH3 domain) are dispensable for synaptic function," said Dr. Bai.

The authors first tested the effect of deleting the synaptojanin PRD, proposed to mediate its interaction with endophilin, on synaptic transmission. Strikingly, worms expressing PRD-deficient synaptojanin in the absence of wild-type synaptojanin showed no defects in synaptic vesicle endocytosis, neurotransmission, or behavior. Simultaneous expression of PRD-deficient synaptojanin and endophilin lacking its SH3 domain in the absence of wild-type versions of either protein fully restored synaptic transmission, strongly arguing that the current model of synaptojanin PRD-endophilin SH3 interaction requires revision.

In addition to the PRD, synaptojanin contains two catalytic domains that remove phosphates from phosphoinositide membrane lipids: Sac1 and a central phosphoinositide phosphatase

domain. Expression of synaptojanin carrying a mutation in the central phosphatase domain that abolishes its phosphatase activity completely abrogated its ability to rescue neurotransmission and behavioral defects in worms lacking wild-type synaptojanin. In contrast, mutation of the Sac1 domain had no such effect, consistent with the observation that humans with mutations in the synaptojanin Sac1 domain show no severe symptoms for the first few decades of life.

While the authors found that the phosphatase activity of the Sac1 domain was dispensable for synaptic transmission, they wondered if it might be playing another role. They thus expressed Sac1-deleted synaptojanin in the absence of wild-type protein and found that this form of the protein did not rescue synaptic and behavioral deficits. By analyzing synapses microscopically, they found that the Sac1 domain was required for targeting of synaptojanin to synapses. To test this further, the authors tethered Sac1-deleted synaptojanin to endophilin. Indeed, this bypassed the requirement for the Sac1 domain, rescuing the defects in behavior and neurotransmission of synaptojanin mutant worms. This effect was dependent on the endophilin BAR domain.

"Our study reveals a novel mechanism that coordinates the action of two essential proteins (synaptojanin and endophilin) for synaptic vesicle endocytosis, which significantly changes the current thinking in the field," said Dr. Bai. "The functional connection between synaptojanin Sac1 and endophilin BAR is highly intriguing because BAR proteins bend membranes during endocytosis. It suggests that the activity of synaptojanin is linked to the transient morphology of endocytic membranes. Together, these surprising findings revise our understanding of how synaptojanin and endophilin support brain activity, and have important ramifications for the field." The roles of synaptojanin and endophilin also appear to extend to other tissues. For instance, an isoform of synaptojanin increases cell migration and invasion in cell culture as well as metastasis of breast tumor xenografts in mice. Thus, much remains to be learned about these intriguing proteins.

Dong Y, Gou Y, Li Y, Liu Y, Bai J. 2015. Synaptojanin cooperates *in vivo* with endophilin through an unexpected mechanism. *eLife*10.7554/eLife.05660.

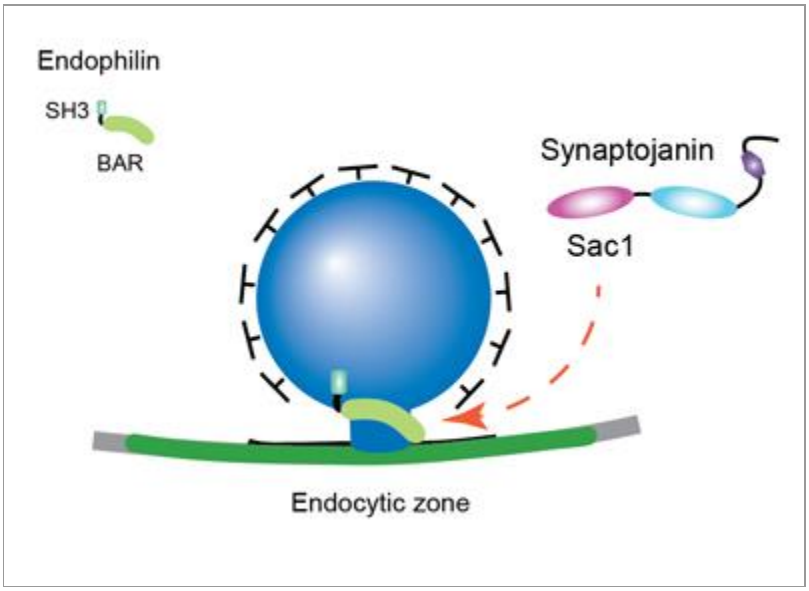

Image provided by Dr. Jihong Bai

Schematic showing cooperation between synaptojanin Sac1 and endophilin BAR at membranes to support synaptic vesicle recycling.